

FORM PTO-1390 (REV 10-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER FJIN-107	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				U.S. APPLICATION NO. (known, do 37 CFR 1.5) 09/787397	
INTERNATIONAL APPLICATION NO. PCT/JP99/05080		INTERNATIONAL FILING DATE September 17, 1999		PRIORITY DATE CLAIMED September 17, 1998	
TITLE OF INVENTION PREVENTIVE AND/OR THERAPEUTIC FOR OBESITY					
APPLICANT(S) FOR DO/EO/US Masaaki Goto, Akihiro Tomoyasu, Kyoji Yamaguchi, Masahiko Kinosaki, Nobuaki Nakagawa					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
1. <input checked="" type="checkbox"/> This is a submission of items concerning a filing under 35 U.S.C. 371.					
2. <input type="checkbox"/> This is a or submission of items concerning a filing under 35 U.S.C. 371.					
3. <input checked="" type="checkbox"/> This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).					
4. <input type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).					
5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))					
a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau).					
b. <input checked="" type="checkbox"/> has been communicated by the International Bureau.					
c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).					
6. <input checked="" type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).					
7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))					
a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau).					
b. <input type="checkbox"/> have been communicated by the International Bureau.					
c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.					
d. <input checked="" type="checkbox"/> have not been made and will not be made.					
8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).					
9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (unexecuted)					
10. <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).					
11. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.					
12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.					
13. <input checked="" type="checkbox"/> A FIRST preliminary amendment.					
<input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.					
14. <input type="checkbox"/> A substitute specification.					
15. <input type="checkbox"/> A change of power of attorney and/or address letter.					
16. <input checked="" type="checkbox"/> Other items or information:					
a. Copy of Request Form (PCT/RO/101) in Japanese and English (8 pages)					
b. Statement Regarding Sameness and New Matter with Regards to the CRF and the Substitute Sequence Listing					
c. Substitute Sequence Listing Disk in computer readable form					
d. Substitute Sequence Listing					
e. Copy of International Search Report dated December 21, 1999					
f. International Deposit Receipt Form BP-6736 in Japanese and English					
g. English translation of International Preliminary Examination Report					

09/787397

532 Rec'd PCT/PTO 16 MAR 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#3/a

Applicant : GOTO et al.
Serial No. : U.S. National Stage Application Of PCT/JP99/05080 Group Art Unit:
Filed : Concurrently herewith Examiner:
Title : PREVENTIVE AND/OR THERAPEUTIC FOR OBESITY

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

With reference to the above-identified patent application, please amend the application prior to issuance of the first Office Action:

IN THE SPECIFICATION

Please replace the Sequence Listing in the English translation of PCT/JP99/05080, as filed, with the Substitute Sequence Listing filed concurrently herewith.

IN THE CLAIMS

Please add new claims 2-20, as follows:

2. A preventive and/or therapeutic agent for obesity according to claim 1 wherein said stanniocalcin is obtained from a recombinant expression cell.
3. A preventive and/or therapeutic agent for obesity according to claim 2 wherein

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Preliminary Amendment

said cell is an animal cell.

4. A preventive and/or therapeutic agent for obesity according to claim 1 which is administered to human beings and animals.

5. A preventive and/or therapeutic agent for obesity according to claim 1 in the form of a pharmaceutical composition.

6. A preventive and/or therapeutic agent for obesity according to claim 5 wherein said pharmaceutical composition is formulated for oral or parenteral administration.

7. A preventive and/or therapeutic agent for obesity according to claim 5 wherein said pharmaceutical composition is selected from the group consisting of compositions for injection, compositions for dripping, suppositories, nasal agents, sublingual agents and percutaneous absorption agents.

8. A preventive and/or therapeutic agent for obesity according to claim 5 wherein said pharmaceutical composition comprises pharmaceutically acceptable carriers, excipients, stabilizers, coloring matters, or surfactants.

9. A preventive and/or therapeutic agent for obesity according to claim 5 wherein said pharmaceutical composition is a composition formulated for injection comprising a pharmacologically effective amount of stanniocalcin and at least one pharmaceutically acceptable excipient and/or activator.

10. A preventive and/or therapeutic agent for obesity according to claim 9 wherein said excipient and/or activator is one or more of amino acids, human serum

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albumin, sugars and/or cellulose derivatives.

11. A preventive and/or therapeutic agent for obesity according to claim 1 which is administered at a daily dose of 10 μ g to 10 mg/kg body weight.

12. A preventive and/or therapeutic agent for obesity according to claim 6 which is administered intravenously.

13. A method for treating or preventing obesity which comprises administering to humans or animals a pharmaceutically effective amount of stanniocalcin in the form of a pharmaceutical composition.

14. A method for treating or preventing obesity according to claim 13 wherein said pharmaceutical composition is administered orally or parenterally.

15. A method for treating or preventing obesity according to claim 13 wherein said pharmaceutical composition is in a form selected from the group consisting of compositions for injection, compositions for dripping, suppositories, nasal agents, sublingual agents and percutaneous absorption agents.

16. A method for treating or preventing obesity according to claim 13 wherein said pharmaceutical composition further comprises pharmaceutically acceptable carriers, excipients, stabilizers, coloring matters, or surfactants.

17. A method for treating or preventing obesity according to claim 13 wherein said pharmaceutical composition is a composition formulated for injection comprising a pharmacologically effective amount of stanniocalcin and at least one pharmaceutically

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acceptable excipient and/or activator.

18. A method for treating or preventing obesity according to claim 17 wherein said excipient and/or activator comprise amino acids, human serum albumin, sugars, and/or cellulose derivatives.

19. A method for treating or preventing obesity according to claim 13 wherein said pharmaceutical composition is administered at a daily dose of 10 μ g to 10 mg/kg body weight.

20. A method for treating or preventing obesity according to claim 13 wherein said pharmaceutical composition is administered intravenously.

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Support for the Amendment

Support for the Substitute Sequence Listing, filed concurrently herewith, can be found, for example, in the Sequence Listing in the English Translation of PCT/JP99/05080, as filed, at pages 1-5. Amendments to the Sequence Listing are solely editorial in nature. The Substitute Sequence Listing contains no new matter.

Support for new claims 2 and 3 can be found, for example, in the instant specification at page 5, lines 11-17. Support for new claims 4, 5 and 13 can be found, for example, in the instant specification at page 6, lines 3-6. Support for new claims 6 and 14 can be found, for example, in the instant specification at page 6, lines 7-8. Support for new claims 7 and 15 can be found, for example, in the instant specification at page 6, lines 8-12. Support for new claims 8 and 16 can be found, for example, in the instant specification at page 6, lines 12-16. Support for new claims 9 and 17 can be found, for example, in the instant specification at page 6, lines 16-22. Support for new claims 10 and 18 can be found, for example, in the instant specification at page 6, lines 16-22, and page 14, lines 12-20. Support for new claims 11 and 19 can be found, for example, in the instant specification at page 7, lines 3-5. Support for new claims 12 and 20 can be found, for example, in the instant specification at page 7, lines 5-7. Upon the entry of this Preliminary Amendment, claims 1-20 will stand pending in the instant application. No new matter is added by the instant Amendment.

Based on the above, an early and favorable action on the merits is earnestly

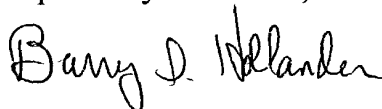
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solicited.

Filed concurrently herewith, Applicants have submitted a Substitute Sequence Listing and a diskette in a computer-readable form.

If any fee is due, please charge our deposit account no. 501032.

Respectfully submitted,



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March 16, 2001

Enclosure: "Versions with Markings to Show Changes Made"

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Version with Markings to Show Changes Made

New claims 2-20 have been added.

PCT

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特許協力条約に基づいて公開された国際出願



(51) 国際特許分類6 A61K 38/17, 38/22	A1	(11) 国際公開番号 WO00/16795 (43) 国際公開日 2000年3月30日(30.03.00)
<p>(21) 国際出願番号 PCT/JP99/05080</p> <p>(22) 国際出願日 1999年9月17日(17.09.99)</p> <p>(30) 優先権データ 特願平10/263004 1998年9月17日(17.09.98) JP</p> <p>(71) 出願人 (米国を除くすべての指定国について) 雪印乳業株式会社 (SNOW BRAND MILK PRODUCTS CO., LTD.)(JP/JP) 〒065-0043 北海道札幌市東区苗穂町6丁目1番1号 Hokkaido, (JP)</p> <p>(72) 発明者 ; および</p> <p>(75) 発明者 / 出願人 (米国についてのみ) 後藤雅昭(GOTO, Masaaki)(JP/JP) 〒329-0511 栃木県下都賀郡石橋町下古山456-1 Tochigi, (JP) 友保昌拓(TOMOYASU, Akihiro)(JP/JP) 〒329-0519 栃木県下都賀郡石橋町大松山1-3-3 SKマンション3-E Tochigi, (JP) 山口京二(YAMAGUCHI, Kyoji)(JP/JP) 〒330-0006 埼玉県大宮市島町702-12 ライオンズガーデン東大宮1-524 Saitama, (JP)</p>		<p>木野崎雅彦(KINOSAKI, Masahiko)(JP/JP) 〒329-0528 栃木県河内郡上三川町ゆうきが丘53-8 Tochigi, (JP) 中川信明(NAKAGAWA, Nobuaki)(JP/JP) 〒329-0415 栃木県下都賀郡国分寺町川名子31-1 ハイツサカエE201 Tochigi, (JP)</p> <p>(74) 代理人 藤野清也(FUJINO, Seiya) 〒160-0004 東京都新宿区四谷1丁目2番1号 三浜ビル8階 Tokyo, (JP)</p> <p>(81) 指定国 AU, CA, CN, HU, JP, KR, NO, NZ, US, ZA, 欧州 特許 (AT, BE, CH, DE, DK, ES, FI, FR, GB, IE, IT, LU, NL, SE)</p> <p>添付公開書類 国際調査報告書</p>
<p>(54)Title: <u>PREVENTIVES AND/OR REMEDIES FOR OBESITY</u></p> <p>(54)発明の名称 肥満予防及び／又は治療剤</p> <p>(57) Abstract Novel preventives and/or remedies for obesity. These preventives and/or remedies contain as the active ingredient stanniocalcin. Because of having an excellent activity of inhibiting the differentiation and maturation of adipocytes, they are useful as drugs for preventing and/or treating obesity.</p>		

DESCRIPTION

PREVENTIVE AND/OR THERAPEUTIC FOR OBESITY

Technical Field

The present invention relates to a novel preventive and/or therapeutic for obesity.

A pharmaceutical preparation of the present invention has excellent preventive and/or therapeutic effects for obesity and is useful as a pharmaceutical.

Background Art

Obesity is a risk factor of diseases such as diabetes mellitus, hypertonia, and heart disease, which threaten health of people in advanced countries. Obesity means physical conditions wherein adipose tissues have abnormally accumulated. Adipose tissues are special organs wherein surplus in vivo energies are stored as fat or triglyceride, and constructed of fibroblasts including adipocytes and their precursors, macrophages, blood vessel surrounding cells, blood cells, and the like.

Adipocytes are said to amount from 1/3 to 2/3 of cells which are present in adipose tissues and to accumulate fats or triglycerides therein. Adipocytes differentiate and mature through the process starting from mesenchymal multipotent stem cells, and growing into lipoblasts which have acquired a base as adipocytes, precursor adipocytes with no lipid droplets but

having initial markers of adipocytes, immaturated adipocytes containing lipid droplets, and finally into matured adipocytes containing a large quantity of accumulated fats. Adipocytes of adults suffering from slight obesity hypertrophically grow due to increase in the amount of accumulated triglyceride. Number of adipocytes increases as the degree of obesity becomes conspicuous. Therefore, decreasing the number of adipocytes by controlling differentiation and maturation or suppressing hypertrophia of matured adipocytes are expected to stop progress of obesity by suppressing the increase in the amount of accumulated fats, and to treat obesity. Control of in vivo adipocyte differentiation has been proven to undergo either positively or negatively according to a number of factors derived from environmental factors such as ingestion, exercise, and so on. As cytokines which control differentiation of adipocytes from adipocyte precursors, tumor necrosis factor- α (TNF- α : Torti F. M. et al., Science, Vol. 229, p 867 (1985)), transforming growth factor- β (Ignotz R. A. et al., Proc. Natl. Acad. Sci. USA, Vol. 82, p 8530 (1985)), preadipocyte factor-1 (Pref-1: Smas C.M. et al., Cell, Vol. 73, p 725 (1993)), and the like have been reported. In addition, leptin, the translational product of an ob gene which has recently been cloned, has been reported to possibly decrease the intake amount and the weight of adipose tissues via central nerve

system (Levin N. et al. Proc. Natl. Acad. Sci. USA, Vol. 93. P 1726, 1996) .

Furthermore, intracerebral peptide-neuropeptide Y which exhibits a strong appetite stimulating effect and its receptor are gathering attention as materials for the development of an obesity suppressing pharmaceutical (Sainsburg A. et al, Diabetologia, Vol.39, p353, 1996). These cytokines are expected to become a therapeutic agent for obesity due to their adipocyte depressing action on accumulation of fat. Clinical tests as an obesity therapeutic or preventive agent is ongoing on some of these cytokines such as leptin.

At present, one obesity therapeutic or preventive agent is commercially available in the USA under the Redux™ (American Home Products Co.). Other drugs such as Meridia (Kunol Co.) and Xenical (Roche Co.) will be approved as an obesity treating agent or a fat absorption inhibitor in the USA. The treatments method using these pharmaceuticals, however, are not necessarily satisfactory in the effects and therapeutic results. Development of a new agent which is available exhibits for these pharmaceuticals higher curative effect and less side effect usable have been desired.

Disclosure of the Invention

In view of the above circumstances, the present inventors have intensive investigated a substance which shows

anti-obesity activity or obesity-curing activity, and as a result, found that stanniocalcin (STC: Olsen H.S. et al., Proc. Natl. Acad. Sci. USA, vol. 93, p 1792 (1996)) which is known as a protein controlling metabolism of minerals, exhibits adipogenesis inhibitory activity, or inhibitory activity of differentiation and/or maturation of adipocytes, which physiologic activity has not been expected of stanniocalcin at all in the past. Accordingly, the object of the present invention is to provide a preventive and/or therapeutical agent for obesity containing a novel substance as an effective ingredient. The present invention relates to a preventive and/or therapeutical agent for obesity, which contains stanniocalcin as an effective ingredient. The pharmaceutical preparation according to the present invention can exhibit excellent preventive and/or therapeutic agent effects for obesity and are useful as a pharmaceutical.

Stanniocalcin was discovered in fishes at first and was subsequently clarified to exist in mammals including humans. Then, cDNA of human embryo was isolated by the genetic engineering procedure on the basis of structural similarity. Human stanniocalcin can be obtained by expressing the resultant cDNA in a variety of cells using the genetic engineering technique.

It has been well known that stanniocalcin reduces a calcium level *in vivo* when given to fish, and also inhibits phosphate excretion to urine when administered to rats (Proc. Natl. Aca. Sci. USA., 93, 1792 (1996)). However, stanniocalcin has not been known to possess excellent preventive and therapeutic effects for obesity.

The Best Mode for Carrying out the Invention

Stanniocalcin, or the effective ingredient according to the present invention, can be obtained by the method of Olsen H. S. et al. (Proc. Natl. Acad. Sci. USA, vol. 93, p 1792 (1996)). Specifically, the above-described literature reference or gene bank or the like can be searched to learn the sequence of cDNA of stanniocalcin, and based on the sequence information, stanniocalcin cDNA can be obtained using the PCR method etc.. The stanniocalcin expression cell can be obtained by transfections of the expression vector into animal cells etc., the said expression vector is obtained by insertion of the resultant cDNA. Then, stanniocalcin can be obtained by cultivating the resultant stanniocalcin expression cells, followed by purification of the resultant culture solution by conventionally employed procedures. The adipogenesis inhibitory activity can be determined by estimating the suppression effects of adipogenesis induced by dexamethasone with retardation of triglyceride accumulation using mouse preadipocytic cell as a target according to the

method of Kodama H. et al. (Journal of Cellular Physiology, Vol. 112, p83 (1982)),

Stanniocalcin, or the effective ingredient of the present invention, can be safely administered to human being and animals in the form of pharmaceutical compositions intended for use in the prevention and/or treatment of obesity. Stanniocalcin can be made into pharmaceutical preparations and administered either for orally or parenterally. Examples of the pharmaceutical composition include compositions for injection, compositions for dripping, suppositories, nasal agent, sublingual agent, percutaneous absorption agent, and the like. These pharmaceutical preparations are formulated according to known pharmaceutical preparation methods using pharmaceutically acceptable carriers, excipients, stabilizers, coloring matters, surfactants and other additives, and made into target preparations. In the case of compositions for injection, a pharmacologically effective amount of stanniocalcin, which is the effective ingredient of the present invention, may be mixed with pharmaceutically acceptable excipients/activators, such as amino acids, sugars, cellulose derivatives and other organic/inorganic compounds, which may be generally added to compositions for injection. If necessary, pH adjusting agents, buffer agents, stabilizers, solubilizing agents, etc. may be added to thereby make a

variety of injectable solutions in accordance with the conventional procedures.

Administration thereof is normally done to human adults at a daily dose of 10 µg to 10 mg/kg body weight, as divided in several times, either orally or parenterally. The particularly preferred dosage form is intravenous administration.

Example

Examples given in the below describe the present invention in more detail, whereby these examples are merely illustrative, and the present invention is in no way understood to be limited by them.

Example 1

Production of stanniocalcin

i) Isolation of poly(A)+ RNA from IMR-90 cells (pulmonary fibroblasts of human embryo, ATCC CCL-186)

About 10 µg of poly(A)+ RNA was isolated from 1×10^8 of IMR-90 cells using Fast Track mRNA Isolation Kit (Invitrogen Inc.) according to the protocol of Invitrogen Inc..

ii) Construction of human stanniocalcin expression vector

A single-stranded cDNA was synthesized using SuperScript II cDNA synthesis Kit (Gibco BRL Inc.) and 1 µg of the isolated poly(A)+ RNA used as a template, according to the protocol of Gibco BRL Inc.. Stanniocalcin (STC) cDNA fragment

was obtained by carrying out PCR using the obtained cDNA template and primer STCF1N (Sequence Identification No. 1) and primer STCR1Xh (Sequence Identification No. 2) as designed according to the nucleotide sequence of human stanniocalcin.

The composition for PCR solution is as follows:

10X Ex Taq Buffer (Takara Shuzo Co.)	10 μ l
2.5 mM dNTP	8 μ l
cDNA solution	1 μ l
Ex Taq (Takara Shuzo Co.)	0.5 μ l
Distilled water	74.5 μ l
20 μ M Primer STCF1N	5 μ l
100 μ M Primer STCR1Xh	1 μ l

The above-described solutions were mixed in a microcentrifugal tube, and PCR was performed under the following conditions: after pretreatment at 95°C for 3 min, the reaction of the three steps of at 95°C for 30 sec, at 55°C for 30 sec and at 72°C for 2 min was repeated 30 times. Then, the reaction mixture incubated at 70°C for 5 min. A portion of the reaction mixture was subjected to agarose gel electrophoresis, and a uniform DNA fragment of about 900 bp was identified. The fragment was sequenced by the conventional method, and it was confirmed to obtain the cDNA encoding stanniocalcin gene. The cDNA sequence and the amino acid sequence are shown in

Sequence Identification Nos. 3 and 4, of sequence table respectively.

The resultant DNA fragment of about 900 bp was purified using QIAEXII DNA extraction kit (QIAGEN Inc.), and the purified DNA was cleaved by restriction enzymes XhoI and NheI (Takara Shuzo Co.) and purified using QIAEXII DNA extraction kit (STC XhoI-NheI fragment). Plasmid pCEPSTC which contained DNA encoding stanniocalcin gene was obtained by ligating the STC XhoI-NheI fragment to pCEP4 (Invitrogen Inc.) cleaved by restriction enzymes XhoI and NheI by ligation kit ver. 2 (Takara Shuzo Co.). *E. coli* (DH5 α ; Gibco BRL Inc.) containing the plasmid has been deposited, in the name of DH5 α /pCEP-STC and under Accession No. FERM BP-6736 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, The Ministry of International Trade and Industry, located at 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan (postal code 305-8566) on May 31, 1999. No erroneous uptake of bases in the DNA portion derived from PCR during the DNA synthesis was meanwhile confirmed by DNA sequencing.

iii) Expression of human stanniocalcin

E. coli DH5 α having pCEPSTC as obtained in Example 1-ii) was cultivated with shaking in 2 ml of Teriffic Broth (Life Technologies Inc.) containing 50 μ g/ml of ampicillin (Sigma

Inc.) and 4.7 % of glycerol overnight at 37°C, and the plasmid DNA was purified from the bacterial cells using QIAWELL kit (QIAGEN Inc.). 293-EBNA cells (Invitrogen Inc.) in IMDM (Life Technologies Inc.) containing 10 % of fetal bovine serum were seeded in each well of a 24-well plate to 2×10^5 /well/ml, followed by cultivation in a CO₂ incubator (5 % CO₂) at 37°C overnight. pCEPSTC or pCEP4 was transfected to 293-EBNA cells using Fugene 6 (Behringer Mannheim Co.). DNA and Fugene 6 were used in portions of 0.5 µg and 1 µl, respectively. After transfection, the transfected cells were cultivated in a CO₂ incubator (5 % CO₂) at 37°C for 3 days. The resultant culture solution was assayed for adipogenesis inhibitory activity by the below-described procedure.

iv) Determination of adipogenesis inhibitory activity

Adipogenesis inhibitory activity was determined by the following procedure according to the method of Kodama H. et al. (Journal of Cellular Physiology, Vol. 112, p 83 (1982)): that is, using mouse pre-adipocytic cell strain MC3T3-G2/PA6 (RIKEN GENE BANK, RCB1127) as a target cell, the adipogenesis induced by dexamethasone was determined with triglyceride accumulation as an index of its inhibitory activity. The culture solution from the sample (Example 1-iii)) diluted with α-MEM (Gibco BRL Inc.) containing 10 % of fetal bovine serum, the culture solution of cells having the vector alone transfected, and the

culture solution of pure 293-EBNA cells were distributed in each portion of 50 μ l into 96-well microplate, respectively, and 3×10^3 cells of pre-adipocytic cell strain MC3T3-G2/PA6 after being suspended in 50 μ l of α -MEM containing 2×10^{-7} M of dexamethasone and 10 % of fetal bovine serum were seeded, followed cultivation at 5% CO₂, 37°C and 100% humidity for one week. After cultivation for 7 days, the culture medium was removed by aspiration, then air-dried and assayed for the triglyceride accumulated in adipocytes with use of a triglyceride measuring kit (Triglyceride G-Test Wako, Code No. 274-69802, Wako Pure Chemicals Ind. Co.). The decreases at OD 510 nm were used for assessment of adipogenesis inhibitory activity. The obtained results are shown in Table 1. As the result, stanniocalcin in the resultant culture solution was confirmed to exhibit adipogenesis inhibitory activity.

Table 1:

Dilution	1/4	1/8	1/16	1/32
Culture solution of STC gene-transfected cells	0.061	0.060	0.057	0.054
Culture solution of vector-transfected cells	0.036	0.021	0.009	0.007
Culture solution of	0.032	0.017	0.014	0.011

293-EBNA cells

Example 2

Determination of adipogenesis inhibitory activity using cells of mouse preadipocytic cell strain 3T3/L1

Using mouse pre-adipocytic cell strain 3T3-L1 (deposited at ATCC-Accession No. CL173) as a target, the formation of adipocytes induced by dexamethasone and 1-methyl-3-isobutylxanthine was measured by means of triglyceride accumulation, to determine the suppressing activity against adipocyte formation. Specifically, 50 μ l of a sample equivalent to the one in Example 1 diluted with α -MEM (Gibco BRL Inc.) containing 10 % of fetal bovine serum was placed into a 96-well microplate, and 5×10^3 cells of mouse pre-adipocyte 3T3-L1 were suspended in 50 μ l of α -MEM containing 4×10^{-7} M of dexamethasone, 2×10^{-5} M of 1-methyl-3-isobutylxanthine and 10 % of fetal bovine serum and then seeded, followed by cultivation at 5% CO₂, 37°C and 100% humidity for one week. After cultivation for 7 days, the culture medium was removed by aspiration, and the cells were air-dried to measure the triglyceride accumulated in adipocytes using a triglyceride assay kit (Triglyceride G-Test Wako, Code No. 274-69802, Wako Pure Chemicals Ind. Co.). The

decrease of OD at 510 nm was taken as adipogenesis inhibitory activity. The obtained results are shown in Table 2. As a result stanniocalcin in the culture solution was confirmed to exhibit adipogenesis inhibitory activity, as in Example 1, when 3T3-L1 cells are used as a target.

Table 2:

Dilution	1/4	1/8	1/16	1/32
Culture solution of STC gene-transfected cells	0.081	0.083	0.082	0.083
Culture solution of vector-transfected cells	0.026	0.017	0.012	0.011
Culture solution of 293-EBNA cells	0.021	0.004	0.006	0.016

Example 3

Pharmaceutical Preparation Examples

Pharmaceutical Preparation Example 1: Production of Injection preparation

One mg of stanniocalcin obtained in Example 1 and 50 mg of human serum albumin were dissolved in 100 ml of 0.01M phosphate buffer solution (PBS, pH 7.0), and the solution was sterilized, divided into vials (2 ml each), lyophilized and sealed.

Pharmaceutical Preparation Example 2: Production of Injection preparation.

Fifty mg of stanniocalcin obtained in Example 1, 1 mg of Tween 80 and 50 mg of human serum albumin were dissolved in

100 ml of 0.01M phosphate buffer solution (PBS, pH 7.0), and the solution was sterilized, divided into vials (2 ml each), lyophilized and sealed.

Pharmaceutical Preparation Example 3: Production of Injection preparation.

One hundred mg of stanniocalcin obtained in Example 1, 50 mg of human serum albumin and 4 g of sorbitol were dissolved in 20 ml of 0.01M phosphate buffer solution (PBS, pH 7.0), and the solution was sterilized, divided into vials, lyophilized and sealed.

Industrial Applicability

According to the present invention, there is provided a novel preventive and/or therapeutic for obesity, which contains stanniocalcin as an effective ingredient. The pharmaceutical preparation of the present invention can exhibit excellent preventive and/or therapeutic effects against obesity and is useful as a pharmaceutical.

Reference to the Deposited Microorganisms

a. Name and address of the Depository organization in which the relevant microorganisms were deposited:

Name: National Institute of Bioscience and Human-
Technology, Agency of Industrial Science and
Technology, The Ministry of International Trade
and Industry

Address: 1-3 Higashi 1-chome, Tsukuba-shi, Ibaraki-ken,
Japan (postal code 305-8566)

- b. The date when deposit was made with the organization of
 - a. May 31, 1999 (as transferred from Bikoken No. P-16933, which was deposited on August 11, 1998).
- c. Accession Number attached to the deposit by the organization of a.

FERM BP-6736

CLAIM

1. A preventive and/or therapeutic agent for obesity which contains stanniocalcin as an effective ingredient.

ABSTRACT

To provide a novel preventive and/or therapeutic agent for obesity.

The preventive and/or therapeutic agent for obesity contains as an active ingredient stanniocalcin.

The preventive and/or therapeutic agent of the present invention has an excellent activity of inhibiting the differentiation and maturation of adipocytes, thereby being useful as a drug for preventing and/or treating obesity.

#6

Please type a plus sign (+) inside this box → ☐

PTO/SB/01 (12-97)

Approved for use through 9/30/00 OMB 0651-0032

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63) <input type="checkbox"/> Declaration Submitted with Initial Filing OR <input checked="" type="checkbox"/> Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)	Attorney Docket Number	FJIN-107
	First Named Inventor	GOTO
	COMPLETE IF KNOWN	
	Application Number	09 / 787,397
	Filing Date	03/16/2001
	Group Art Unit	
	Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

PREVENTIVE AND/OR THERAPEUTIC FOR OBESITY

the specification of which (Title of the Invention)

☐ is attached hereto
OR
☒ was filed on (MM/DD/YYYY) 03/16/2001 as United States Application Number or PCT International Application Number 09/787,397 and was amended on (MM/DD/YYYY) 03/16/2001 (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
263004/1998	Japan	09/17/1998	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
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			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below

Application Number(s)	Filing Date (MM/DD/YYYY)

☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

[Page 1 of 2]

Burden Hour Statement. This form is estimated to take 0.4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231

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DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
PCT/JP99/05080	09/17/1999	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith ☐ Customer Number ☐ OR ☒ Registered practitioner(s) name/registration number listed below

Place Customer Number Bar Code Label here

Name	Registration Number	Name	Registration Number
Barry I. Hollander	28,566	Andrew E. C. Merriam	47,268

☐ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto

Direct all correspondence to: ☐ Customer Number or Bar Code Label 023290 OR ☒ Correspondence address below

Name	Barry I. Hollander				
Address	Hollander Law Firm, P.L.C.				
Address	Suite 305, 10300 Eaton Place				
City	Fairfax	State	VA	ZIP	22030
Country	United States	Telephone	703-383-4800	Fax	703-383-4804

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle (if any))		Family Name or Surname			
Masaaki		Goto			
Inventor's Signature	<i>Masaaki Goto</i>			Date	4/27/2001
Residence: City	Shimotsuga-gun, Tochigi	State	JPX	Country	Japan
Post Office Address	456-1, Shimokoyama, Ishibashi-machi				
Post Office Address					
City	Shimotsuga-gun, Tochigi	State		ZIP	329-0511
				Country	Japan

☒ Additional inventors are being named on the 2 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

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PTO/SB/02A (3-97)
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DECLARATION

ADDITIONAL INVENTOR(S) Supplemental Sheet Page 1 of 2

Name of Additional Joint Inventor, if any:				<input type="checkbox"/> A petition has been filed for this unsigned inventor			
Given Name (first and middle [if any])				Family Name or Surname			
<u>Akihiro</u>				<u>Tomoyasu</u>			
Inventor's Signature	<u>Akihiro Tomoyasu</u>			Date	<u>4/27, 2001</u>		
Residence: City	Shimotsuga-gun, <u>Tochigi</u>	State	<u>JPX</u>	Country	Japan	Citizenship	Japan
Post Office Address	SK-Mansion 3-E, 1-3-3, Omatsuyama, Ishibashi-machi						
Post Office Address							
City	Shimotsuga-gun, <u>Tochigi</u>	State		ZIP	329-0519	Country	Japan
Name of Additional Joint Inventor, if any:				<input type="checkbox"/> A petition has been filed for this unsigned inventor			
Given Name (first and middle [if any])				Family Name or Surname			
<u>Kyoji</u>				<u>Yamaguchi</u>			
Inventor's Signature	<u>Kyoji Y.</u>			Date	<u>4/27, 2001</u>		
Residence: City	Omiya-shi, <u>Saitama</u>	State	<u>JPX</u>	Country	Japan	Citizenship	Japan
Post Office Address	Lionsgarden Higashiomiya 1-524, 702-12 Shimacho						
Post Office Address							
City	Omiya-shi, <u>Saitama</u>	State		ZIP	330-0006	Country	Japan
Name of Additional Joint Inventor, if any:				<input type="checkbox"/> A petition has been filed for this unsigned inventor			
Given Name (first and middle [if any])				Family Name or Surname			
<u>Masahiko</u>				<u>Kinosaki</u>			
Inventor's Signature	<u>Masahiko Kinosaki</u>			Date	<u>4/27, 2001</u>		
Residence: City	Kawachi-gun, <u>Tochigi</u>	State	<u>JPX</u>	Country	Japan	Citizenship	Japan
Post Office Address	53-8, Yuukigaoka, Kaminokawa-machi						
Post Office Address							
City	Kawachi-gun, <u>Tochigi</u>	State		ZIP	329-0528	Country	Japan

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DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 2 of 2

Name of Additional Joint Inventor, if any:				<input type="checkbox"/> A petition has been filed for this unsigned inventor			
Given Name (first and middle [if any])				Family Name or Surname			
<u>Nobuaki</u>				<u>Nakagawa</u>			
Inventor's Signature	<u>Nobuaki Nakagawa</u>			Date	<u>4/27/2001</u>		
Residence: City	Shimotsuga-gun, <u>Tochigi</u>	State	<u>JPX</u>	Country	Japan		
Post Office Address Heights Sakae E201, 31-1, Kawanago, Kokubunji-machi							
Post Office Address							
City	Shimotsuga-gun, Tochigi	State		ZIP	329-0415	Country	Japan
Name of Additional Joint Inventor, if any:				<input type="checkbox"/> A petition has been filed for this unsigned inventor			
Given Name (first and middle [if any])				Family Name or Surname			
Inventor's Signature				Date			
Residence: City		State		Country			
Post Office Address							
Post Office Address							
City		State		ZIP		Country	
Name of Additional Joint Inventor, if any:				<input type="checkbox"/> A petition has been filed for this unsigned inventor			
Given Name (first and middle [if any])				Family Name or Surname			
Inventor's Signature				Date			
Residence: City		State		Country			
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